[redacted]

23. The method of claim 22 further comprising the step of subsequently withdrawing the second stimulus, thereby achieving reduction of activity or inactivation of the gene of interest.

24. A nucleic acid delivered into a cell comprising a gene for a transcription factor that is operably linked to a promoter activatable by a stress and by the transcription factor.

25. A nucleic acid delivered into a cell comprising a first gene for a transcription factor that is operably linked to a promoter activatable by a stress and a second gene for the same transcription factor that is operably linked to a promoter activatable by the transcription factor.

26. A nucleic acid delivered into a cell comprising a gene for a transcription factor, the transcription factor being first expressed in response to a stress and thereafter activating its own expression.

27. The nucleic acid of any of claims 24-26, wherein the transcription factor is selected from the group consisting of a mutated heat shock transcription factor, a chimeric transcription factor, a constitutively active transcription factor and a transcription factor active in the presence of a second stimulus other than a stress.

REMARKS

Rejection of claims 9-18 under 35 U.S.C. 112, first paragraph

Examiner rejected all pending claims under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner observed that "the claims as written are not limited to such a mutant HSF and encompass any transcription factor that is stress-induced". The Examiner then commented on the activation (of HSF), which involves a complex, multistep process, citing inventors own published art, and remarked

that "the specification does not provide any guidance on how to predictably manipulate or mutate undiscovered and undisclosed transcription factors so that they will stay in the active form once stimulated (by a transient stress)". Applicant realizes that his proposed claims unintentionally encompassed unspecified transcription factors that are activated in response to a transient stress. What he intended to include as a claim element was a/any transcription factor whose expression (synthesis) is induced by a transient stress. To remedy this problem, Applicant proposes new claims 19-27. For example, new claim 19 reads as follows:

> 19. A molecular regulatory circuit delivered into a cell for activation of a gene of interest by a single transient stress comprising a gene for a transcription factor and the gene of interest, whereby the transcription factor is first expressed in response to the transient stress and thereafter activates transcription of the gene of interest and its own expression.

The language of this claim makes it clear that it is the synthesis of the transcription factor that is induced by a transient stress, and that the transcription factor, once made, is capable of stimulating its own expression as well as that of the gene of interest.

In the preliminary remarks that accompanied the submission of the present divisional patent application, Applicant stated as reason for the submission his wish to better protect what he regarded as his invention by characterizing and claiming his molecular circuits using functional language. The discussion that follows is intended explain why Applicant believes to be entitled to the proposed new claims.

The specification separates the molecular circuits of the invention into three classes that are discussed in turn. In type 1 circuits, the transcription factor is a mutated heat shock transcription factor (HSF). Therefore, both transcription factor gene and gene of interest must be controlled by a stress-inducible promoter (the target of the transcription factor). Thus, in the special case of the type 1 circuits, expression of the product of the gene of interest is not only stimulated by the transcription factor but also transiently occurs in

response to an activating stress. Type 1 circuits are described on p.12, lines 11-19 and in Figure 1: "In the cells, both genes (mutated transcription factor gene and gene of interest) are either silent or expressed at appropriately low levels in the absence of stress. When the cells are stressed, promoters in both elements are activated by endogenous HSF, which results in the expression and accumulation of gene product of interest and of mutated HSF. Mutated HSF continues to activate transcription of both the gene of interest and its own gene, resulting in the synthesis of more product of interest and mutated HSF. This cycle continues even if the cells are no longer under stress. Consequently, the gene of interest will remain active until such time that the cell has exhausted its capacity to transcribe and translate nucleic acids". Claim 19 captures the essential features of type 1 circuits: it specifies that the circuits comprise two elements, a transcription factor gene and a gene for a protein of interest. It further characterizes the circuits by requiring that the transcription factor is first expressed in response to a transient stress and thereafter activates transcription of the gene of interest and its own expression, which results in maintenance of gene activity. Because the claim is intended to cover all three types of circuits, the specific features of type 1 circuits that are absent from type 3 circuits, i.e., that the gene of interest is also responsive to the initial transient stress and that the transcription factor is a mutated HSF, are not present as limitations in the claim.

In the type 2 circuits, the transcription factor is a mutated HSF that additionally contains a different DNA-binding domain from its normal domain, which interacts with HSE sequences in heat shock promoters. Type 2 circuits are described on p.20, lines 3-8 and, additionally, by the example in Figure 2: "After transient stress, endogenous HSF is activated, which results in expression and accumulation of mutated HSF. During and subsequent to stress, mutated HSF activates the promoter controlling the gene of interest as well as its own promoter, resulting in expression of the protein of interest as well as additional mutated HSF. The continued expression of mutated HSF stimulates synthesis of the protein of interest until the cell's capacity is exhausted". Claim 19 captures the essential features of type 2 circuits, which circuits contain two elements, a gene for a transcription factor, i.e., a particular mutated HSF, and a gene of interest. The gene for the transcription factor is first activated by a transient stress, resulting in the expression

and accumulation of the transcription factor. The transcription factor then activates the gene of interest and supports its own expression. Because the claim is intended to cover all three types of circuits, the specific feature of type 2 circuits that is absent from type 3 circuits, i.e., that the transcription factor is a mutated HSF, is not present as a limitation in the claim.

Claim 19 also captures a subset of type 3 circuits that are identical to type 2 circuits except that the transcription factor is not a mutated heat shock transcription factor, but a (any) constitutively active transcription factor. This subset of type 3 circuits is characterized on p.21, lines 16-28, and is illustrated in Figure 3: "Other forms of circuits can be constructed in which the transcription factor is not mutated HSF. That is, any constitutively active transcription factor can be used in lieu of mutated HSF. A type 3 circuit is illustrated in Figure 3. Its elements are first a construct containing a gene for a constitutively active transcription factor, here the synthetic factor LexA-activation domain, linked to a promoter activatable by both a stress and the transcription factor. Transcription factor LexA-activation domain contains the DNA-binding and dimerization domains of bacterial repressor LexA fused to a transcriptional activation domain. Alternatively, two constructs, contained in one or two nucleic acids, can be substituted. One of the constructions contains the transcription factor gene linked to a stress promoter, and the other the transcription factor gene linked to a promoter responsive to the transcription factor. The second element is a construct containing a gene of interest controlled by a promoter responsive to the transcription factor".

In the other subset of type 3 circuits, the transcription factor is a transcription factor that is active in the presence of a second stimulus (other than a stress). Such type 3 circuits are described on p. 21, lines 29-30, on p.22, lines 1-26, and in Fig.4. The operation of such circuits is explained on the example of an EcR/RXR heterodimeric transcription factor, whose activity requires the presence of a hormone ligand such as muristerone A. In the specific example, two sets of transcription factor subunit genes are provided, one of which is regulated by a stress-inducible promoter and the other by a promoter responsive to the heterodimeric transcription factor. It is noted that the use of two transcription factor

constructs instead of one in which the transcription factor gene(s) is activatable by both a stress and the transcription factor is a variation of the circuits that is specifically discussed for type 3 circuits on p.21, lines 23-26.

On p.22, lines 17-26, it is provided that "this form of type 3 circuit operates as follows. In the absence of either one or both, stress and hormone, the gene of interest is silent. In the presence of hormone, a transient stress will activate on set of transcription factor genes, resulting in accumulation of (transcription factor) EcR/RXR that is activated by hormone. Active factor will activate expression from the second set of transcription factor genes as well as the gene of interest. Because the transcription factor is continually produced, activation of the gene of interest is sustained. Upon withdrawal of the hormone, transcription factor is inactivated, and the autoactivating loop is interrupted. The gene of interest is no longer expressed, and the inactive transcription factor as well as the protein of interest will eventually be degraded by intracellular proteolytic systems". Figure 4 illustrates the same mode of operation graphically. Thus, in its simplest version captured in claim 20, this form of type 3 circuits comprises two elements, a gene for a transcription factor that is activated by a second stimulus and a gene of interest. The transcription factor is expressed (made) in response to a transient stress and acquires activity in the presence of the second stimulus. It is then capable of activating the gene of interest and its own expression.

Claims 21 and 22 recast the composition of matter claims 19 and 20 as methods claims. It is obvious that the methods comprise the two steps of first delivering the circuit and then activating it. See, for example, p.12, lines 10-13. Claim 23 is dependent on claim 22 and claims the additional step of withdrawal of the second stimulus for inactivation of the circuit. Support for this step is found on p.22, lines 23-26. Claim 26 is a composition of matter claim directed to the novel transcription factor element of the molecular circuits of the invention. Similar language is used to characterize this composition that was used to characterize the molecular circuits in claim 19. Claims 24 and 25 relate to alternative forms of the composition of claim 26, cast in language identical to that used to characterize the molecular circuits in claims 1 and 9 of the parent application 09/304,121.

Support for the elements of dependent claim 27 can also be found in the specification. For "mutated heat shock transcription factor" see p.12, lines 8, 14 and 16, and p.14, lines 14 and 17. For "chimeric transcription factor" see the definition on p.9, lines 3-6, and the examples on p.19, lines 15-20, p.20, lines 21-28 (The factors discussed are specifically called "chimeras" on line 27.), and p.21, lines 21-23. For "constitutively active transcription factor" see p.21, lines 17-18. For "a transcription factor active in the presence of a second stimulus other than a stress" see the example of p.22, lines 4-9 and line 20. For the characterization of the second stimulus as being different from a stress see the sentence bridging pp. 21 and 22. In light of the substitution of claims 9-18 with new claims 19-27 and the explanations provided above, Examiner is respectfully requested to withdraw her rejection under 35 U.S.C. 112, first paragraph.

Double patenting

Claims 9-18 were rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-36 of U.S. patent No. 6,342,596. Applicant believes that the double patenting rejection would similarly apply to new claims 19-27. Applicant includes with this response a fully executed terminal disclaimer and fee. Consequently, Examiner is requested to withdraw her double patenting rejection.

Applicant believes that substitution of new claims 19-27 for old claims 9-18 and execution of a terminal disclaimer as requested will place the application in condition for allowance. Examiner is cordially invited to call Applicant at (305) 243-5815 if he believes that a further discussion would be helpful for advancing this case.

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Respectfully Submitted,

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